

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 16/46, A61K 39/00, 39/395		A1	(11) International Publication Number: WO 97/43316
			(43) International Publication Date: 20 November 1997 (20.11.97)
(21) International Application Number: PCT/US97/07707		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 6 May 1997 (06.05.97)		Published <i>With international search report.</i>	
(30) Priority Data: 60/017,249 10 May 1996 (10.05.96) US 08/841,815 5 May 1997 (05.05.97) US			
(71) Applicant: BETH ISRAEL DEACONESS MEDICAL CENTER, INC. [US/US]; West Campus, One Deaconess Road, Boston, MA 02215 (US).			
(72) Inventor: JUNGHANS, Richard, P.; 47 Rutland Square #3, Boston, MA 02118 (US).			
(74) Agents: LOREN, Ralph, A. et al.; Lahive & Cockfield, L.L.P., 28 State Street, Boston, MA 02109 (US).			
(54) Title: PHYSIOLOGICALLY ACTIVE MOLECULES WITH EXTENDED HALF-LIVES AND METHODS OF USING SAME			
(57) Abstract			
<p>The present invention is drawn to physiologically active molecules which have extended half-lives in the circulatory system of a subject, compositions which include these molecules, methods of producing the molecules, and methods of using the molecules to treat subjects. The half-lives of the physiologically active molecules are extended by modifying their structure such that they are capable of binding to the IgG protection receptor FcRp. By modifying the physiologically active molecules in this manner, the invention takes advantage of the discovery that the FcRp and the FcRn are the same receptor and that modifying physiologically active molecules such that they are capable of binding the IgG protection receptor FcRp allows these molecules to escape lysosomal catabolism and remain in the circulation of a subject for longer periods of time.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	St. Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

PHYSIOLOGICALLY ACTIVE MOLECULES WITH EXTENDED HALF-LIVES AND METHODS OF USING SAME

Background of the Invention

5 This invention relates to methods for extending the half-life of physiologically active molecules in subjects to whom the molecules are administered. These methods include modifying physiologically active molecules such that they include regions or domains which can bind to selected receptors, e.g., the FcRp protection receptor, which recycle the molecules to the circulation rather than allowing the molecules to pass to lysosomal catabolism.

10 Thirty-two years ago in an article published in the journal *Nature*, F.W.R. Brambell disclosed a hypothesis to explain IgG catabolism which has remained the cornerstone in our understanding of IgG catabolism ever since. Brambell et al. (1964) *Nature* 203:1352-1355. To explain the correlation of high IgG levels with increased catabolism (Humphrey and Fahey (1961) *J. Clin. Invest.* 40:1696-1705; Sell and Fahey (1964) *J. Immunol.* 93:81-87;
15 Sell, S.(1964) *J. Exp. Med.* 120:967-986), Brambell proposed that specific IgG "protection receptors" (FcRp) bind IgG in pinocytotic vacuoles and redirect IgG transport to the circulation. In addition, according to Brambell, when the FcRp are saturated, unbound IgG is allowed to pass to unrestricted lysosomal catabolism.

 In other studies, Dr. Brambell also described a second receptor, the neonatal gut
20 transport receptor (FcRn), which is found in neonatal gut and placenta. Brambell (1966) *Lancet* 2:1087-1093; Waldmann and Strober (1969) *Progr. Allergy* 13:1-110; Jones and Waldmann (1972) *J. Clin. Invest.* 51:2916-2927; Waldmann and Jones (1972) in Protein Turnover, Ciba Foundation Symposium 9:5-18. Recently, the FcRn has been cloned and characterized (Simister and Mostov (1989) *Nature* 337:184). FcRn was found to be a
25 heterodimer of a membrane-integral class I-like heavy chain and a β -2 microglobulin (β_2m) light chain (Simister and Mostov (1989) *Nature* 337:184), in which both chains make essential contacts with Fc (Burmeister et al. (1994) *Nature* 372:279-383). When Fc is mutated in the domains contacting either FcRn heavy or light chain (Burmeister et al. (1994) *Nature* 372:279-383), survival and transport are both adversely affected (Kim et al. (1994)
30 *Eur. J. Immunol.* 24:2429-2434). In mice deleted for light chain ($\beta_2m^{-/-}$), FcRn surface expression is lost and neonatal pups are devoid of maternal IgG transport (Israel et al. (1995) *J. Immunol.* 154:6246-6251). The same study noted that older $\beta_2m^{-/-}$ mice had autologous IgG levels 1/10th those of normal mice. This observation was proposed by the authors to reflect decreased IgG synthesis.

35 Over the years, it has been determined that the FcRp and the FcRn receptor systems have several common features. For example, the two receptors mediate transendosomal

- 2 -

transport of IgG and can both be saturated with IgG. Brambell (1964) *Nature* 203:1352-1355; Humphrey and Fahey (1961) *J. Clin. Invest.* 40:1696-1705; Sell and Fahey (1964) *J. Immunol.* 93:81-87; Sell (1964) *J. Exp. Med.* 120:967-986; Brambell (1966) *Lancet* 2:1087-1093; Waldmann and Strober (1969) *Progr. Allergy* 13:1-110; Jones and Waldmann (1972) *J. Clin. Invest.* 51:2916-2927; Waldmann and Jones (1972) in Protein Turnover, Ciba Foundation Symposium 9:5-18. In addition, the two receptors share an Fc binding site (Kim et al. (1994) *Eur. J. Immunol.* 24:2429-2381; Burmeister et al. (1994) *Nature* 372:379-383) and enhanced IgG binding under acidic conditions (Brambell (1966) *Lancet* 2:1087-1093; Waldmann and Strober (1969) *Progr. Allergy* 13:1-110; Jones and Waldmann (1972) *J. Clin. Invest.* 51:2916-2927; Waldmann and Jones (1972) in Protein Turnover, Ciba Foundation Symposium 9:5-18; Story et al. (1994) *J. Exp. Med.* 180:2377-2381). In the thirty years since the disclosure of Brambell's hypothesis, however, the FcRp protection receptor has not been identified and the lack of genetic markers to define its activity has made the determination of its identity extremely difficult.

Summary of the Invention

The present invention is based, at least in part, on the discovery that the FcRp and the FcRn are the same receptor. This discovery lead to the recognition that the IgG protective capabilities of the FcRp can be imparted on a variety of therapeutics, e.g., human therapeutics, to extend the half-lives of the therapeutics in a subject. Thus, the present invention is drawn to physiologically active molecules which have extended half-lives in the circulatory system of a subject, compositions which include these molecules, methods of producing the molecules, and methods of using the molecules to treat subjects. The half-lives of these molecules are extended by modifying their structure such that they are capable of binding to the IgG protection receptor FcRp. By modifying the physiologically active molecules in this manner, the invention takes advantage of the discovery that the FcRp and the FcRn are the same receptor and that modifying physiologically active molecules such that they are capable of binding the IgG protection receptor FcRp allows these molecules to escape lysosomal catabolism and remain in the circulation of a subject for longer periods of time.

The physiologically active molecules which are modified according to the invention include molecules which can bring about a desired result in a subject. For example, physiologically active molecules can be human therapeutics or human drugs for which an extended half-life is beneficial to a subject, e.g., a human patient. Non-limiting examples of physiologically active molecules include proteins and peptides, e.g., immunoglobulins, immunoglobulin fragments or portions, e.g., all or a portion of IgG3, IgA, IgD, IgE, IgM, and

- 3 -

other nonimmunoglobulin molecules including cytokines, e.g., TGF- β , interleukin-2, interleukin-10, interleukin-12, GM-CSF, and vaccine immunogens, e.g., gp120 for HIV, HBSAg of hepatitis B, hemagglutinin of influenza virus, coat protein of respiratory syncytial virus, tetanus toxoid of *C. tetani*, outer membrane proteins of *P. pneumoniae*, *V. cholerae*, *S. typhae*, *L. monocytogenes*, and *M. tuberculosis*. These physiologically active molecules can be used to treat neoplastic diseases, viral diseases such as AIDS, and other immune diseases and infections.

The half-lives of the physiologically active molecules of the invention, e.g., drugs, e.g., drugs for human therapy, are increased by modifying the structure of the molecules in a manner which allows them to bind to the IgG protection receptor FcRp. For example, the structure of the physiologically active molecules can be modified to include a specific amino acid sequence which binds to the IgG protection receptor FcRp but which does not bind to an Fc receptor which mediates immune effects or which binds to the IgG protection receptor FcRp but which does not bind to complement. In one embodiment, the specific amino acid sequence is substantially homologous, e.g., at least about 60%, 70%, 80%, 90%, 95%, 98%, or 99% or more homologous, to an amino acid sequence of at least a portion of the IgG domain which binds to the IgG protection receptor FcRp. In a preferred embodiment, the amino acid sequence is the same as the amino acid sequence of at least a portion of an IgG domain which binds to the IgG protection receptor FcRp.

In another embodiment, the physiologically active molecules are proteins or peptides and the structure of these molecules is altered by amino acid substitution such that the protein or peptide specifically binds to the IgG protection receptor FcRp.

In yet another embodiment, the structure of the physiologically active molecule can be modified to include at least a portion of an antibody which is raised against the IgG protection receptor FcRp and which specifically binds to the IgG protection receptor FcRp. In a preferred embodiment, the antibody or portion or fragment thereof binds to the IgG protection receptor at acidic pH, e.g., a pH of about 6.5 or lower, and is released from the IgG protection receptor at neutral pH, e.g., a pH of about 7.0 to about 7.5. The portion or fragment of the antibody can be a Fab fragment, an Fv fragment, a Fab' fragment or a F(ab')₂ fragment.

Another aspect of the invention pertains to compositions which include the physiologically active molecules described herein together with a pharmaceutically acceptable carrier.

Yet another aspect of the invention pertains to methods for producing a physiologically active molecule which has an extended half-life in the circulatory system of a subject. These methods include modifying the structure of the molecule such that it includes a specific amino acid sequence which binds to the IgG protection receptor FcRp but which does not bind to an Fc receptor which mediates immune effects or which binds to the IgG

- 4 -

protection receptor FcRp but which does not bind to complement. In one embodiment, the specific amino acid sequence is substantially homologous, e.g., at least about 60%, 70%, 80%, 90%, 95%, 98%, or 99% or more homologous, to an amino acid sequence of at least a portion of the IgG domain which binds to the IgG protection receptor FcRp. In a preferred
 5 embodiment, the specific amino acid sequence is the same as the amino acid sequence of at least a portion of an IgG domain which binds to the IgG protection receptor FcRp.

A still further aspect of the invention pertains to methods for producing a physiologically active protein or peptide having an extended half-life in the circulatory system of a subject. These methods include altering the amino acid sequence of the protein
 10 or peptide by amino acid substitution such that the protein or peptide specifically binds to the IgG protection receptor FcRp. In one embodiment, the protein or peptide is an immunoglobulin or fragment or portion thereof, e.g., all or a portion IgG3, IgA, IgD, IgE and IgM.

The invention also pertains to methods for producing a physiologically active molecule having an extended half-life in the circulatory system of a subject which includes
 15 modifying the structure of the molecule such that it includes at least a portion of an antibody, e.g., a Fab, Fv, Fab' or F(ab')₂ fragment, which is raised against the IgG protection receptor FcRp and which binds to the IgG protection receptor FcRp. In a preferred embodiment, the antibody or portion or fragment thereof binds to the IgG protection receptor at acidic pH, e.g.,
 20 a pH of about 6.5 or lower, and is released from the IgG protection receptor at neutral pH, e.g., a pH of about 7.0 to about 7.5.

The invention further pertains to methods for treating a subject having a disorder which can be treated with a physiologically active molecule. These methods include
 25 administering to the subject the physiologically active molecules or compositions described herein.

Brief Description of the Drawings

Figure 1 is a graph which depicts IgG survival over time in wild-type (■) and in $\beta_2m^{-/-}$ (FcRp-) (□) mice. This figure demonstrates that, over time, IgG survival is greater in wild-
 30 type mice than in $\beta_2m^{-/-}$ (FcRp-) mice.

Figure 2 is a graph which depicts IgG half-lives with increasing IgG concentrations in wild-type (■) and $\beta_2m^{-/-}$ (FcRp-) (□) mice. This figure demonstrates that with increasing IgG concentration, IgG survival is suppressed in wild-type mice but not in $\beta_2m^{-/-}$ (FcRp-) mice.

Figures 3A-3B are graphs which depict the survival in wild-type (Figure 3A) or in $\beta_2m^{-/-}$ (FcRp-) (Figure 3B) mice of soluble Tac antigen (human IL2R α) plus excess
 35

- 5 -

nonspecific antibody (--Δ--) and soluble Tac antigen plus specific antibody (—Δ—). Also shown in Figures 3A and 3B is the survival of specific antibody (anti-Tac (■)). These figures demonstrate the loss of differential catabolism mechanism for antigen-in-complex and antibody-in-complex in $\beta_2m^{-/-}$ (FcRp-) mice.

5

Detailed Description of the Invention

In one aspect, the invention pertains to physiologically active molecules having extended half-lives in the circulatory system of a subject due to modification of the molecules' structures to include a specific amino acid sequence which binds to the IgG protection receptor FcRp but which does not bind to an Fc receptor which mediates immune effects or which binds to the IgG protection receptor FcRp but which does not bind to complement. As used herein, the term "physiologically active molecule" refers to a molecule which has at least one biological activity when administered to a subject. In a preferred embodiment, the biological activity of the physiologically active molecule has a beneficial effect, e.g., decreases symptoms of a disorder or prevents infection by an infectious agent, on the subject to whom it is administered. Physiologically active molecules include proteins, peptides, lipids, carbohydrates, nucleic acids, inorganic molecules and molecules which are combinations thereof. In a preferred embodiment, the physiologically active molecule is a protein or peptide. Non-limiting examples of physiologically active molecules include proteins and peptides, e.g., immunoglobulins, immunoglobulin fragments or portions, e.g., all or a portion of IgG3, IgA, IgD, IgE, and IgM, cytokines such as TGF- β , interleukin-2, interleukin-10, interleukin-12, GM-CSF, and vaccine immunogens such as gp120 for HIV, HBSAg of hepatitis B, hemagglutinin of influenza virus, coat protein of respiratory syncytial virus, tetanus toxoid of *C. tetani*, outer membrane proteins of *P. pneumoniae*, *V. cholerae*, *S. typhae*, *L. monocytogenes*, and *M. tuberculosis*. These physiologically active molecules can be used to treat neoplastic diseases, viral diseases such as AIDS, and other immune diseases and infections as described herein.

Subjects to whom the physiologically active molecules can be administered include mammals. In a preferred embodiment, the subjects are humans. The circulatory or cardiovascular systems of the subject include the heart and blood vessels (arteries, arterioles, capillaries, venules, veins, and sinuses) and the lymphatic system.

The term "half-life" as used herein refers to the time required to eliminate one-half of the quantity of the physiologically active molecule that was present in the circulatory system of a subject at the point when the measurement was begun. The term "extended half-life" when used herein to refer to the modified physiologically active molecules of the invention is intended to include physiologically active molecules, modified according to the invention,

35

- 6 -

which have half-lives which are longer than the half-lives of the physiologically active molecules prior to modification according to the invention.

As used herein, the phrase "specific amino acid sequence" refers to an amino acid sequence which includes amino acid residues capable of binding to the FcRp but which do not bind to an Fc receptor which mediates immune effects or which are capable of binding to the FcRp protection receptor but which do not bind to complement. Typically, the Fc receptors which mediate immune effects are located on immune cells. As used herein, the term "immune cell" is intended to include a cell which plays a role in specific immunity (e.g., is involved in an immune response) or plays a role in natural immunity. Examples of immune cells include all distinct classes of lymphocytes (T lymphocytes, such as helper T cells and cytotoxic T cells, B lymphocytes, and natural killer cells), monocytes, macrophages, other antigen presenting cells, dendritic cells, and leukocytes (e.g., neutrophils, eosinophils, and basophils). Fc receptors on immune cells are surface receptors which specifically bind to the Fc fragment of IgG. See e.g., Unanue, E.R. and Benacerraf, B. Textbook of Immunology, Second Edition (Williams & Wilkins, Baltimore, MD, 1984) 92, 111, 112. The Fc receptors (e.g., FcRI, FcRII, and FcRIII) on immune cells are not involved in protection of IgG from catabolism. These Fc receptors do, however, mediate diverse immune effects or effector functions. The term "immune effects" as used herein refers to effects, activities, or functions mediated, directly or indirectly, by an immune cell. These immune effects or effector functions include, but are not limited to regulation of immunoglobulin production, lysosomal enzyme release, tumor necrosis factor α release, interleukin-1 and interleukin-6 release, interferon γ release, cell-mediated cytotoxicity by immune cells against cells that bind the Fc-containing molecule. van Winkel and Capel (1993) *Immunol. Today* 14(5):215-220. It is specifically contemplated in the present invention that the physiologically active molecules described herein are capable of binding to the FcRp to thereby take advantage of its protective effects but do not bind to an Fc receptor which mediates immune effects. Thus, the physiologically active molecules of the invention have increased survival in the recipient subject but do not initiate immune effects which can be detrimental to the subject to whom the molecules are administered. As used herein, the term "complement" is an art recognized term which refers to a system (or a molecule or molecules within the system) of functionally linked proteins, portions, or complexes thereof which interact with one another in a regulated manner to provide many of the effector functions of humoral immunity and inflammation. See Abbas et al. Cellular and Molecular Immunology (W.B. Saunders Company, Philadelphia, 1991) pp. 259-282. Nonlimiting examples of molecules which are part of the complement system include C1, C1q, C1r, C1s, C2, C3, C5, C6, C7, C8, C9, Factor B, and Factor D.

- 7 -

Thus, in one embodiment, the specific amino acid sequence described herein can include amino acid sequences found in IgG which are involved in binding to the FcRp protection receptor but which do not bind to Fc receptors which mediate immune effects. For example, as the FcRI, FcRII, FcRIII, and complement binding activities of IgG are located in the CH2 domain (amino acids 321 to 240) near the hinge and are not involved in FcRp protection receptor binding, these sites are either not included as specific amino acid sequences in the physiologically active molecules or, if they are included as specific amino acid sequences in the physiologically active molecules, they are altered to delete the binding domains for FcRI, FcRII, FcRIII, and complement. If Leu (amino acid 234) and Pro (amino acid 331) of the Fc domain of IgG are changed to different amino acid residues, IgG binding to FcRI is abolished. Canfield and Morrison (1991) *J. Exp. Med.* 173:1483. In another example, if Glu (amino acid 318), Lys (amino acid 320), and Lys (amino acid 322) of the Fc domain of IgG are changed to different amino acid residues, binding of IgG to complement is abolished. Duncan and Winter (1988) *Nature* 332:738; Sandie and Michaelson (1991) *Mol. Immunol.* 28:1361. In addition, there are binding sites for FcRI, FcRII, and FcRIII of the Fc domain of IgG which overlap. Sarmay et al. (1992) *Mol. Immunol.* 29:633. Thus, when the amino acid sequence of these binding sites are altered, binding to FcRI, FcRII, and FcRIII can be altered, e.g., abolished, simultaneously.

As demonstrated herein, the FcRp protection receptor is the same receptor as the FcRn receptor which has previously been cloned and characterized. See e.g., Story et al. (1994) *J. Exp. Med.* 180:2377-2381; Simister and Mostov (1989) *Nature* 337:184; Burmeister et al. (1994) *Nature* 372:279-383; Kim et al. (1994) *Eur. J. Immunol.* 24:2429-2434; and Israel et al. (1995) *J. Immunol.* 154:6246-6251. Although it is stated herein that the FcRp protection receptor and the FcRn receptor are the same receptor, it is specifically contemplated by the invention that the FcRp protection receptor can include differences in the nucleic acid encoding the receptor and/or amino acid sequence of the receptor from the nucleic acid encoding the FcRn receptor and/or amino acid sequence of the FcRn receptor wherein the differences do not affect the ability of the physiologically active molecules of the invention to bind to the FcRp protection receptor. Thus, both the nucleic acid and amino acid sequences of the FcRp protection receptor are also available to the skilled artisan. Moreover, regions of the IgG molecule which are involved in binding to the FcRn receptor have also been identified. See Burmeister et al. (1994) *Nature* 372:379-383. These regions include amino acid residues 248 to 257 (KTLMIS RTP) (SEQ ID NO:1); amino acid residue 272 (E); amino acid residue 285 (H); amino acid residue 288 (K); amino acid residues 290 to 291 (KP); amino acid residues 308 to 311 (VLHQ) (SEQ ID NO:2); amino acid residue 314 (L); amino acid residues 385 to 387 (GQP); amino acid residue 428 (M); and amino acid residues 433 to

436 (HNHY) (SEQ ID NO:3). These regions form a motif which is referred to herein as the Brambell motif. Thus, the physiologically active molecules, e.g., proteins and peptides, of the invention can be modified as described herein to include at least a portion of an amino acid sequences which is substantially homologous, e.g., at least about 60%, 70%, 80%, 90%, 95%, 98%, or 99% or more homologous, to an amino acid sequence, e.g., the Brambell motif, of at least a portion of the IgG domain which binds to the IgG protection receptor FcRp.

Amino acid sequences which are substantially homologous to an amino acid sequence of at least a portion of the IgG domain which binds to the IgG protection receptor FcRp can include amino acid sequences which are the same as at least a portion of the IgG domain with the exception of at least one conservative amino acid substitution which does not alter or only slightly alters the ability of the amino acid sequence to bind to the FcRp protection receptor. In a preferred embodiment, the specific amino acid sequence is the same as the amino acid sequence of at least a portion of an IgG domain which binds to the IgG protection receptor FcRp.

The term "homologous", as used herein, refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two polypeptide molecules is occupied by alanine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For example, if 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the amino acid sequences Asp-Ala-Glu-Gly-His-Tyr and Val-Ala-Val-Gly-Thr-Tyr share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology. As used herein, the term "substantially homologous" refers to a polypeptide having an amino acid sequence at least about 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous to at least a portion of an region or domain, e.g., IgG region or domain, e.g., the Brambell motif, which binds to the IgG protection receptor FcRp. In a preferred embodiment, the polypeptide includes an amino acid sequence essentially the same as at least a portion of an amino acid sequence of an IgG region or domain which binds to the IgG protection receptor FcRp.

In another aspect, the invention pertains to physiologically active proteins or peptides having an extended half-life in the circulatory system of a subject and which have a structure which has been altered by amino acid substitution such that the protein or peptide specifically binds to the IgG protection receptor FcRp. Methods of altering the amino acid sequence of the physiologically active molecules, e.g., proteins and peptides, are known in the art and

described herein. For example, to obtain additional amino acid sequences of IgG domains which bind to the IgG protection receptor, IgG nucleic acid sequences encoding amino acid sequences involved in or potentially involved in binding to the IgG protection receptor can be mutagenized and the resulting mutant gene products can be screened using the methods described herein. For example, amino acid sequence variants of a molecule, e.g., a protein or peptide, can be prepared by random mutagenesis or non-random (directed mutagenesis) of DNA which encodes the molecule or a particular domain or region of a polypeptide. Examples of random mutagenesis methods include PCR mutagenesis (see e.g., Leung et al. (1989) *Technique* 1:11-15) and saturation mutagenesis (see e.g., Mayers et al. (1985) *Science* 229:242). A library of random amino acid sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotide sequences. Non-random mutagenesis can be used to create variants which include, e.g., deletions, insertions, or substitutions, of residues of the known amino acid sequence of a protein or peptide. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conserved amino acids and then with more radical choices depending upon results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3. Other methods of performing non-random mutagenesis include alanine scanning mutagenesis (see, e.g., Cunningham and Wells (1989) *Science* 244:1081-1085), oligonucleotide-mediated mutagenesis (see, e.g., Adelman et al. (1983) *DNA* 2:183; Crea et al. (1978) *Proc. Natl. Acad. Sci. USA* 75: 5765), cassette mutagenesis (see, e.g., Wells et al. (1985) *Gene*, 34:315), and combinatorial mutagenesis (see, e.g., Ladner et al., WO 88/06630)

Various techniques are known in the art for screening generated mutant gene products. Techniques for screening large gene libraries often include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the genes under conditions in which detection of a desired activity, e.g., binding to the IgG protection receptor FcRp but no binding to an Fc receptor which mediates immune effects or binding to the IgG protection receptor FcRp but no binding to complement, facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Several techniques are amenable to high through-put analysis for screening large numbers of sequences created, e.g., by random mutagenesis techniques. These techniques include, for example, the use of two hybrid systems, use of display libraries, e.g., a filamentous phage system, LamB system, and the use of *in vitro* systems based on the display of nascent peptide in polysome complexes, to screen candidate molecules.

In yet another aspect, the invention pertains to physiologically active molecules having an extended half-life in the circulatory system of a subject and which have a structure

- 10 -

which is modified to include at least a portion of an antibody which is raised against the IgG protection receptor FcRp and which specifically binds to the IgG protection receptor FcRp. An antibody, or fragment, portion, or derivative thereof, which can be included in the structure of the physiologically active molecules of the invention can be derived from polyclonal antisera containing antibodies reactive with a number of epitopes on the antigen, e.g., the IgG protection receptor FcRp. More preferably, however, monoclonal antibodies are raised against the IgG protection receptor (or FcRn receptor) using the IgG protection receptor FcRp (or FcRn receptor) or a cell having the receptor on its surface as the immunogen. Polyclonal and monoclonal antibodies for use in the invention can be prepared by standard techniques known in the art. For example, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an antigen (e.g., IgG protection receptor FcRp or a portion thereof) or with a cell which expresses the antigen (e.g., on the cell surface) to elicit an antibody response against the antigen in the mammal. Alternatively, tissue or a whole organ which expresses the antigen can be used to elicit antibodies. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with the antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein ((1975) *Nature* 256:495-497) as well as other techniques such as the human B-cell hybridoma technique (Kozbar et al. (1983) *Immunol. Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. (1985) *Monoclonal Antibodies in Cancer Therapy*, Allen R. Bliss, Inc., pages 77-96) can be used. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the antigen and monoclonal antibodies isolated.

Another method of generating specific antibodies, or antibody fragments, reactive against epitopes on an antigen is to screen expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with the antigen (or a portion thereof). For example, complete Fab fragments, V_H regions, Fv regions and single chain antibodies can be expressed in bacteria using phage expression libraries. See, e.g., Ward et al. (1989) *Nature* 341:544-546; Huse et al. (1989) *Science* 246:1275-1281; and McCafferty et al. (1990) *Nature* 348:552-554. Alternatively, the Xenomouse can be used to produce antibodies, or fragments thereof (available from Cell Genesys). Antibodies of the appropriate binding specificity which are made by these techniques can be used to alter an antigen on a donor cell.

- 11 -

A preferred antibody portion or fragment for altering an epitope is a F(ab')₂ fragment. Antibodies can be fragmented using conventional techniques. For example, the Fc portion of an antibody can be removed by treating an intact antibody with pepsin, thereby generating a F(ab')₂ fragment. In a standard procedure for generating F(ab')₂ fragments, intact antibodies
5 are incubated with immobilized pepsin and the digested antibody mixture is applied to an immobilized protein A column. The free Fc portion binds to the column while the F(ab')₂ fragments pass through the column. The F(ab')₂ fragments can be further purified by HPLC or FPLC. F(ab')₂ fragments can be treated to reduce disulfide bridges to produce Fab' fragments.

10 In a preferred embodiment, the antibody or portion thereof binds to the IgG protection receptor at acidic pH, e.g. a pH of about 6.5 or lower, and is released from (or does not bind to) the IgG protection receptor at neutral pH, e.g., a pH of about 7.0 to about 7.5. Thus, the physiologically active molecules modified to include the antibody or portion thereof can escape lysosomal catabolism by remaining bound to the IgG protection receptor at low pH
15 and remain in the circulation of a subject for longer periods of time by being released from the IgG protection receptor at neutral pH.

The invention also includes compositions which include the physiologically active molecules described herein together with a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" is intended to include any and all
20 solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for physiologically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the physiologically active molecule, use thereof in the compositions is contemplated.

25 In another aspect, the invention pertains to methods for producing physiologically active molecules which have an extended half-life in the circulatory system of a subject. These methods include modifying the structure of the molecule such that the molecule is capable of binding to the IgG protection receptor FcRp. The physiologically active molecules can be modified by a variety of methods including, for example, altering the amino acid
30 sequence of the physiologically active molecule as described herein to include amino acid sequences which bind to the appropriate receptor; surface reshaping; coupling of two nucleic acid molecules one of which encodes the physiologically active molecule and another which encodes an amino acid sequence capable of binding to the FcRp to generate a fusion protein (see e.g., Capon et al. (1989) *Nature* 337:525-531; U.S. Patent No. 5,116,964 to Capon and
35 Lasky); fos-jun binding (Kostelny, S.A. et al. (1992) *J. Immunol.* 148:1547-1553) or other protein-protein affinity methods; and chemical linkage of molecules to the physiologically

- 12 -

active molecules by derivatizing the molecule to be linked with an amino reactive group that will cross-link with or to the physiologically active molecule through lysine amino groups on the physiologically active molecule. Cumber, J.A. et al. (1985) *Meth. Enzymol.* 112:207-224.

Yet another aspect of the invention pertains to methods for treating subjects having a disorder which is treatable by administering a physiologically active molecule or a composition of the invention. Disorders which can be treated according to the method of the invention include disorders the detrimental effects of which can be alleviated, minimized, decreased, e.g., temporarily or permanently, or abolished by administration of a physiologically active molecule. Non-limiting examples of such disorders (and, in some cases, the physiologically active molecules which can be used to treat the disorders) include autoimmune disorders such as systemic lupus erythematosus, arthritis, and autoimmune thyroiditis (TGF β and IL-10); immunodeficiencies such as Wiskott-Aldrich syndrome and AIDS (IgM); neoplastic disorders such as melanomas and carcinomas (IL-2), e.g., renal cell carcinoma; disorders resulting from the presence of infectious agents such as viruses (e.g., HIV, hepatitis B virus, influenza virus, respiratory syncytial virus) and bacteria (e.g., *P. pneumoniae*, *V. cholerae*, *S. typhae*) and fungi (e.g., *C. albicans*) and parasites (e.g., *leishmania*); and other bacterial, fungal, and parasitic infections (cytokines such as IL-1, IL-2, IL-12; interferon γ ; tumor necrosis factor; Duffy binding proteins for malaria; and p85 cellular protein for Trypanosomal infection).

This invention is further illustrated by the following Examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated by reference.

EXAMPLES

For the experiments described in the following examples, wild-type and genetic knockout mice (FcRp-) were obtained from The Jackson Labs, Bar Harbor, Maine, with either a mixed C57BL/6 x 129/Ola background or an inbred C57BL/6J background. The mice were raised under low pathogen conditions (Sell and Fahey (1964) *J. Immunol.* 93:81-87; Sell (1964) *J. Exp. Med.* 120:967-986) to yield low endogenous IgG levels.

30 Example I: Abbreviated IgG Survival in $\beta 2m^{-/-}$ Mice

Animals (mixed background $\beta 2m^{-/-}$ or wildtype; Jackson Labs) raised under low pathogen conditions as described above were injected with mixtures of ^{131}I murine anti-Tac antibody (■, wildtype; □, mutant) and ^{125}I murine albumin (Inter-Cell). Anti-Tac is an IgG2a, κ antibody against human IL2R α and is not reactive with any mouse proteins or tissues. Five mice were used per group. Blood was sampled at indicated times and processed for protein-bound counts by trichloroacetic acid precipitation as previously described

(Junghans and Waldmann (1996) *J. Exp. Med.* 183:1587-1602). Kinetic parameters were obtained by two-compartment modeling of the composite data of each group using PCNONLIN 4.2 (SCI, Durham). The reported catabolic rate constant is k_{10} in standard pharmacokinetic nomenclature, \pm fitting error. Catabolic $t_{1/2} = \ln 2/k_{10}$. The reported ratio of catabolic constants is between ^{131}I -IgG and ^{125}I -albumin as internal control. The catabolic $t_{1/2}$ values for IgG were 4.9 ± 0.4 days in wild-type versus 0.45 ± 0.07 days in the mutant mice. The catabolic $t_{1/2}$'s of the beta phase of the curves were longer for both - 8.3 and 0.6 days - but beta phase constants are not appropriate for judging catabolism or steady states. Error bars = ± 1 SE, shown only on last points; other points are similar or less.

- 10 Comparison of the survival of administered IgG in wild-type and $\beta 2\text{m}^{-/-}$ mice confirmed a marked acceleration of clearance in the latter (Figure 1). As shown in Figure 1, compartmental modeling revealed catabolic rate constants of $0.14 \pm 0.01 \text{ dy}^{-1}$ for wildtype (■) and $1.5 \pm 0.12 \text{ dy}^{-1}$ for mutant (□) mice. When normalized to albumin co-administered in these tests, the wildtype mice catabolized IgG at a rate of 0.15 relative to albumin, reflecting an approximate 7-fold relative protection of IgG, whereas the mutant mice catabolized IgG and albumin at identical rates (ratio = 0.97 ± 0.05), hence displaying no protection of IgG. Plasma immunoglobulin levels were assayed, in which IgG was approximately 10-fold lower in mutant than in wild-type mice, as noted previously (Israel et al. (1995) *Immunol.* 154:6246-6251), whereas IgA and IgM were the same between groups.
- 20 This direct relation of increased catabolic rates and decreased blood levels of IgG is compatible with pharmacokinetic predictions. These results thus confirm disruption of the protection receptor (FcRp) that parallels FcRn disruption. Further, these data allow quantitation of the protection by the FcRp; the IgG in the normal mice was recycled through cellular endosomes an average of seven times (relative to albumin) before it was finally catabolized.

Example II: Suppression of Antibody Survival by Increased IgG Concentration in Wild-type but not in $\beta 2\text{m}^{-/-}$ Mice.

- 30 As a corollary of its role in protecting IgG from catabolism, the disruption of the FcRp is predicted to disrupt the classical pattern of decreased IgG survival with higher IgG concentration (Brambell et al. (1964) *Nature* 203:1352-1355; Humphrey and Fahey (1961) *J. Clin. Invest.* 40:1696-1705; Sell and Fahey (1964) *J. Immunol.* 93:81-87; Sell (1964) *J. Exp. Med.* 120:967-986; Brambell (1966) *Lancet* 2:1087-1093; Waldmann and Strober (1969) *Progr. Allergy* 13:1-110).

- 35 To recapitulate the phenomenon of increased catabolism with high IgG concentration, experiments of Sell and Fahey ((1964) *J. Immunol.* 93:81-87; Sell (1964) *J. Exp. Med.*

120:967-968) were adapted and periodic injection into mice of purified human IgG (which competes for the protection receptor equally as mouse IgG) was performed. Animals were injected intraperitoneally with ^{125}I human gammaglobulin (Miles) on schedules to maintain different steady state blood levels of IgG for the duration of the experiment. Mice received 1-
 5 3 intraperitoneal doses of human IgG prior to the intravenous injection of ^{131}I anti-Tac, and 0-8 intraperitoneal doses after the intravenous injection of ^{131}I anti-Tac. Mice were injected intravenously with a single dose of ^{131}I anti-Tac, six hours subsequent to the last prior intraperitoneal dose of human IgG. Blood levels of administered human IgG were determined from counts and ^{125}I -IgG specific activity, and added to the estimated total
 10 murine IgG. The plasma loss kinetics of the administered ^{131}I anti-Tac antibody were analyzed for each group as in Figure 1, except that the beta phase $t_{1/2}$ is shown, which parallels prior representations (Brambell et al. (1964) *Nature* 203:1352-1355; Humphrey, J.J. and Fahey (1961) *J. Clin. Invest.* 40:1696-1705; Sell and Fahey (1964) *J. Immunol.* 93:81-87; Sell (1964) *J. Exp. Med.* 120:967-986) of the wild-type curve. A different experimental
 15 design including more early points (e.g., Figure 1) is required for accurate assessment of catabolic rates; however, the catabolic $t_{1/2}$ values can be inferred from Figure 1 data as 0.6-0.75x the beta phase $t_{1/2}$. Each point represents the average of two to five mice. Error bars are not shown: fitting error of the $t_{1/2}$ was 10% or less, and the mid-quartile range for the IgG concentrations over the duration of the experiments was approximately $\pm 25\%$.

20 The results shown in Figure 2 confirm that the disruption of the FcRp disrupts the classical pattern of decreased IgG survival with higher IgG concentration. In Figure 2, the survival $t_{1/2}$ for wildtype (■) or mutant (□) mice is plotted against plasma concentration of IgG, represented as the sum of human and endogenous murine IgG. Wild-type mice exhibited the expected suppression of IgG survival with increased total IgG whereas the
 25 mutant mice showed no similar effect, with IgG behaving essentially as expected for albumin, which does not share the IgG protection receptor and whose clearance is unaffected by IgG concentration (Brambell et al. (1964) *Nature* 203:1352-1355; Humphrey and Fahey (1961) *J. Clin. Invest.* 40:1696-1705; Sell and Fahey (1964) *J. Immunol.* 93:81-87).

30 Example III: Loss of Differential Catabolism Mechanism for Antigen-in-Complex and Antibody-in-Complex in $\beta 2^{-/-}$ Mice

The following experiment was performed with monomeric antigen-antibody complexes. Five mice were used in each group. 0.3 μg (10pmole) of ^{131}I soluble Tac was mixed with 100 μg (1200 pmole binding site) of non-specific or specific ^{125}I antibody and
 35 injected intravenously. The concentration of specific antibody binding sites ranged from 1200 nM to 300 nM over the duration of the experiment, $\geq 1000\text{x}$ the antibody K_d , and

ensuring that antigen binding is essentially complete. Samples were collected and processed for protein-bound counts as above. Antibody binding prolongs survival of the 33kD Tac protein by blocking renal filtration; antibody survival is unaffected by antigen binding.

The results of this experiment are shown in Figures 3A-3B. Both Figures 3A and 3B show the survival in wildtype (Figure 3A) or $\beta 2m^{-/-}$ (Figure 3B) mice of soluble Tac antigen plus excess non-specific antibody (Δ -) and soluble Tac antigen plus specific antibody (Δ -). Also shown is the survival of specific antibody (anti-Tac (\blacksquare); nonspecific isotype control antibody, UPC, not shown). Error bars = ± 1 SE, shown only on last points; other points are similar or less. The results of Figure 3A confirm the observation of differential catabolism in the wild-type mice described herein, with longer survival of antibody than antigen associated with antibody. This was interpreted as antigen dissociation in the acidic endosome, where Fc-FcR β binding is stabilized, with return of antibody to circulation via the protection receptor, thus "cleansing" the antibody of antigen and harvesting antigen for presentation without antibody destruction, as occurs with mlg on B cells (Mamula and Janeway, C.A. (1993) *Immunol. Today* 14:151). When performed with mutant mice (Figure 3B), bound antigen was cleared at the same accelerated rate as (unprotected) antibody. These results confirm that the protection receptor is central to the differential catabolic mechanism for antigen- and antibody-in-complex.

The survival of Tac-in-complex in wildtype ($t_{1/2}$ 1.7 days) versus $\beta 2m^{-/-}$ mice (0.55 day) suggests that Tac bound to antibody in normal animals recycles through the endosome three times before it is dissociated and passed to the lysosome for catabolism versus eight times for the antibody itself in this experiment. By this model, the survival of other antigens passing through the endosome will depend on antigen-antibody affinity (off-time) at acidic endosomal pH's and on the endosomal transit time, expected to be of the order of a few minutes from data on other recycling receptors (Schmid et al. (1988) *Cell* 52:73-83). The normal off-time for Tac from anti-Tac complexes is $t_{1/2}$ 100 min under physiologic conditions (Robb et al. (1984) *J. Exp. Med.* 160:1126-1146); the endosome environment evidently accelerates this dissociation rate to account for the 50% catabolism of Tac-in-complex on a few brief passages through the cell.

It is notable that the catabolic $t_{1/2}$ of 0.43 ± 0.06 day previously estimated for the 10% non-renal fraction of Tac catabolism (90% is renally filtered) (Junghans and Waldmann (1996) *J. Exp. Med.* 183:1587-1602) approximates the value of 0.45 ± 0.07 day for IgG and albumin in the $\beta 2m^{-/-}$ mice (Figure 1), and is also comparable to the non-renal component of L chain and Fab catabolism (Wochner et al. (1967) *J. Exp. Med.* 126:207) and to total catabolism for IgM (Junghans and Waldmann (1996) *J. Exp. Med.* 183:1587-1602); Waldmann and Strober (1969) *Progr. Allergy* 13:1-110), which is not filtered. This

- 16 -

correspondence suggests that the dispersed pinocytotic activities of virtually all cells capture and process all soluble proteins with equivalent degradative rates unless they are protected by specific mechanisms, as studied here with the FcRp and as available to transferrin and other recycled proteins via their cognate receptors (Schmid et al. (1988) *Cell* 52:73-83).

5 Identity of the intestinal and protection receptors is now supported by a common functional disruption from genetic deletion of a subunit of the molecule. This linking of genetic and functional data is the most concrete evidence that the FcRp and FcRn are one and the same, which, by now, represents the most straightforward interpretation of these accumulated data. As the heavy chain and light chain ($\beta 2m$) subunits are each encoded by
10 single copy genes, the expression of this FcR in these contexts should be regulated from the same loci by temporal and tissue-specific factors. Of these two settings, it is as the IgG protection receptor that this FcR has its broadest and most durable expression.

The unifying feature of the FcRn and the protection receptor is high affinity binding at low pH, present both in bowel and in endosome, and low affinity at normal plasma pH. In
15 the protection setting, IgG is not bound to FcRp on the cell surface at all, but only after IgG is passively internalized by ongoing pinocytosis into endosomes where it is returned to circulation with reversal of binding at neutral physiologic pH. The present studies show that normal IgG catabolism is regulated principally through the Brambell receptor (FcRp) because deletion of a subunit of the receptor renders its catabolism indistinguishable from that of
20 albumin in the same mice.

Example IV: Modification of IgM to Extend Half-Life in Circulatory System of a Subject

Physiologically active molecules, e.g., physiologically active proteins or peptides, of
25 the invention can be generated by modifying their structure to include the sequences, e.g., amino acid sequences, which are involved in IgG binding to the FcRp protection receptor in positions according to computerized or manual spatial analysis to approximate the orientation of the domains of IgG. These amino acid sequences are described herein.

The amino acid sequence of an IgM protein against a microorganism or a cancer is
30 altered to include the amino acid sequences set forth in Table I below. These alterations allow IgM to bind to the IgG protection receptor FcRp but not to an Fc receptor which mediates immune effects. In addition, these alterations do not interfere with the ability of IgM to bind complement. Subsets of these alterations or analogous alterations can also be used to alter the amino acid sequence of IgM to impart thereon the ability to bind to the IgG
35 protection receptor FcRp but not to an Fc receptor which mediated immune effects.

TABLE I

IgM amino acid residue numbers (OU Index)	IgM amino acid sequence	Altered IgM amino acid sequence
353-363	FASIFLTKST (SEQ ID NO:4)	PKTLMIS RTP (SEQ ID NO:5)
377	S	PE
391-396	KTH TNI (SEQ ID NO:6)	HNAKTKP (SEQ ID NO:7)
413-419	ICEDDWN (SEQ ID NO:8)	VLHQDWL (SEQ ID NO:9)
539	A	M
544-547	PNRV (SEQ ID NO:10)	HNHY (SEQ ID NO:3)

The amino acid GQP (IgG amino acids 385-387) is preserved in IgM (amino acids 492-494) and does not require modification.

5

IgM is advantageous for use in treatment of gram negative bacterial sepsis (Ziegler et al. (1991) *N. Engl. J. Med.* 324:429-436) and of infection hypersusceptibility of Wiskott-Aldrich syndrome. As the normal survival of IgM in humans is about five days (Waldmann and Strober (1969) *Progr. Allergy* 13:1-110), treatment using IgM requires redosing on a frequent basis. When IgM is modified by altering its amino acid sequence as shown in Table I, its half-life in a human is increased to about 23 days which is equivalent to the half-life of IgG in a human. This increase in half-life results in a reduced redosing schedule and an approximately 80% savings in IgM.

10

15 **Example V: Modification of IgA to Extend Half-Life in Circulatory System of a Subject**

The amino acid sequence of an IgA protein against a microorganism or a cancer is altered to include the amino acid sequences set forth in Table II below. These alterations allow IgA to bind to the IgG protection receptor FcRp but not to an Fc receptor which mediates immune effects. Subsets of these alterations or analogous alterations can also be used to alter the amino acid sequence of IgM to impart thereon the ability to bind to the IgG protection receptor FcRp but not to an Fc receptor which mediates immune effects.

20

TABLE II

IgA amino acid residue numbers (OU Index)	IgA amino acid sequence	Altered IgA amino acid sequence
260-270	ALEDLLLGSEA (SEQ ID NO:11)	PKTLMISRTP (SEQ ID NO:12)
285	G	PE
301-305	SAVQG (SEQ ID NO:13)	VHNAKTK (SEQ ID NO:14)
327-333	GCAEPWN (SEQ ID NO:15)	VLHQDWL (SEQ ID NO:9)
413-414	EL	Q
459	G	M
464-467	PLAF (SEQ ID NO:16)	HNHY (SEQ ID NO:3)

IgA is advantageous for use in treatment of viral infections. Mazanec et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6901-6905; Levine et al. (1991) *Science* 254:856-860. As the normal survival of IgA in humans is about five days (Waldmann and Strober (1969) *Progr. Allergy* 13:1-110), treatment using IgA requires redosing on a frequent basis. When IgA is modified by altering its amino acid sequence as shown in Table II, its half-life in a human is increased to about 23 days which is equivalent to the half-life of IgG in a human. This increase in half-life results in a reduced redosing schedule and an approximately 80% savings in IgA.

Example VI: Modification of Hepatitis B Surface Antigen to Extend Half-Life in Circulatory System of a Subject

Hepatitis B surface antigen (HBsAg) is used to vaccinate subjects against hepatitis B viral infection. Subjects are vaccinated against hepatitis B viral infection through the administration of repeated doses of HBsAg. HBsAg has a half-life of about three hours in the circulatory system of a subject. To increase the half-life of HBsAg, nucleic acid encoding HBsAg is coupled to a nucleic acid molecule encoding an amino acid sequence which includes the Brambell motif as described herein.

HBsAg is a protein of 400 amino acids with several domains of secondary structure and few glycosylation sites (Neurath et al. (1987) *Microbiol. Sciences* 4:45-51). Seven T and B cell epitopes in HBsAg have been defined. These epitopes are not disrupted when HBsAg

- 19 -

is modified according to the invention. The Brambell motif is inserted into the region encompassed by amino acid residues 60 to 80, which is flanked by β sheets, amino acid residues 220-245, the β turn domain, or amino acid residues 160 to 180, the α helix domain of HBSAg. To improve solubility of HBSAg, the hydrophobic domains of amino acid residues 185 to 200, 255 to 270, and 350 to 400, are removed or substituted. HBSAg modified according to this method has a half-life of about 23 days in a human.

Example VII: Modification of HIV Envelope Protein gp120 to Extend Half-Life in Circulatory System of a Subject

The HIV envelope protein gp120 is a major target for immune responses in HIV infection and can be used as a vaccine immunogen. The half-life of gp120 is about 4 days in the circulatory system of the subject. To increase the half-life of gp120, selected domains are preserved while others are modified. gp120 is 510 amino acids in length with a structure which includes disulfides, glycosylations, and five neutralization domains. The disulfide structure and the neutralization domains are preserved. The IgG domains which interact with the FcRp protection receptor are mainly in two broad bands of about 50 to 70 amino acids each that are exposed with intervening buried β sheets. Disulfide delimited loops accomplish a similar sequence externalization and exposure as that of the IgG domains which interact with FcRp. Loops SS2 and SS3 are, therefore, modified to include the Brambell motif. gp120 modified according to this method has a half-life of about 23 days in a human.

In Example VIII to Example X below, several different small molecules are modified by linking the molecules to a carrier molecule which (1) binds to the FcRp protection receptor but which does not bind to an Fc receptor which mediates immune effects; or (2) binds to the FcRp protection receptor but which does not bind to complement. Typically, the carrier molecule is an amino acid sequence which includes amino acid residues which bind to the FcRp protection receptor but which do not bind to an Fc receptor which mediates immune effects; and/or (2) which bind to the FcRp protection receptor but which do not bind to complement. In one embodiment, a carrier molecule which includes these amino acid residues can be produced by selectively modifying the Fc region of IgG, for example, IgG1. The Fc region of IgG includes the amino acid residues which are involved in binding to the FcRp protection receptor but which also include amino acid residues which are involved in binding to Fc receptors which mediate immune effects and/or amino acid residues which are involved in binding to complement. The Fc region of IgG is then modified to preserve the amino acid residues which are involved in binding to the FcRp protection receptor but to delete, e.g., by substitution, the amino acid residues which are involved in binding to Fc

receptors which mediate immune effects or amino acid residues which are involved in binding to complement. The modified Fc region of IgG which has been converted to a carrier molecule, is then linked to the physiologically active molecule to increase the half-life of the molecule.

5

**Example VIII: Modification of Glycophorin A (GpA) to Extend Half-Life
 in Circulatory System of a Subject**

Glycophorin A (GpA) is the target for *P. falciparum* malarial parasite for red blood cell invasion. Blocking GpA with antibody blocks access of parasite to the red blood cell invasion pathway, but treatment with antibody cannot be used *in vivo* because of danger to the red blood cells from the antibody exposure. The reverse strategy is to block the parasite with a soluble GpA. The half-life of GpA, however, is about one hour. To increase the half-life of GpA to about 23 days, GpA is modified as described above. Specifically, the extracellular domain of GpA, which includes only about 85 amino acids, is expressed as a fusion protein which includes the extracellular domain linked to a carrier molecule as described above.

15

**Example IX: Modification of IL-10 and TGF β to Extend Half-Life in
 Circulatory System of a Subject**

IL-10 and TGF β can abort active autoimmune responses in subjects with autoimmune diseases (Sporn and Roberts (1990) *Cell. Regul.* 1:875-882; Moore et al. (1993) *Ann. Rev. Immunol.* 11:165-190). Both IL-10 and TGF β have short half-lives, about one hour each. To increase the half-lives of these molecules to about 23 days, the molecules are modified by linking thereto a carrier molecule as described above. Specifically, IL-10 (18kD) and TGF β (25kD) are expressed as fusion proteins which include these small molecules linked to a carrier molecule as described above.

20

25

**Example X: Modification of IL-2 to Extend Half-Life in Circulatory System of
 a Subject**

IL-2 has been approved for the treatment of renal cell carcinoma and is additionally effective for the treatment of melanoma and other cancers (Rosenberg et al. (1989) *Ann. Surg.* 210(4):474-485). The short half-life (about one hour) of IL-2 necessitates continuous infusion or repeated intravenous or subcutaneous dosing. To increase the half-life of IL-2 to about 23 days, IL-2 is modified by linking thereto a carrier molecule as described above. Specifically, IL-2 is expressed as a fusion protein which includes IL-2 linked to a carrier molecule as described above.

30

35

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention
5 described herein. Such equivalents are intended to be encompassed by the following claims.

- 22 -

SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

- (i) APPLICANT: Richard P. Junghans
- 10 (ii) TITLE OF INVENTION: PHYSIOLOGICALLY ACTIVE MOLECULES WITH
EXTENDED HALF-LIVES AND METHODS OF USING SAME
- (iii) NUMBER OF SEQUENCES: 16
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD
(B) STREET: 60 State Street, Suite 510
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
20 (F) ZIP: 02109-1875
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
30 (B) FILING DATE:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
35 (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Jean M. Silveri
(B) REGISTRATION NUMBER: 39,030
(C) REFERENCE/DOCKET NUMBER: NER-267-1
40
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617)227-7400
(B) TELEFAX: (617)227-5941
45
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
50 (B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

- 23 -

(v) FRAGMENT TYPE: internal

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Lys Thr Leu Met Ile Ser Arg Thr Pro
1 5

10 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 Val Leu His Gln
1

(2) INFORMATION FOR SEQ ID NO:3:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Asn His Tyr
1

45

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
50 (B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (v) FRAGMENT TYPE: internal

- 24 -

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
Phe Ala Ser Ile Phe Leu Thr Lys Ser Thr
1 5 10

10 (2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
Pro Lys Thr Leu Met Ile Ser Arg Thr Pro
25 1 5 10

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
Lys Thr His Thr Asn Ile
1 5

45 (2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
50 (B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

55

- 25 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5 His Asn Ala Lys Thr Lys Pro
 1 5

(2) INFORMATION FOR SEQ ID NO:8:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

 Ile Cys Glu Asp Asp Trp Asn
 1 5

25 (2) INFORMATION FOR SEQ ID NO:9:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
30 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: peptide

35 (v) FRAGMENT TYPE: internal

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

 Val Leu His Gln Asp Trp Leu
 1 5

45 (2) INFORMATION FOR SEQ ID NO:10:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
50 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

55

- 26 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Pro Asn Arg Val
1

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Leu Glu Asp Leu Leu Leu Gly Ser Glu Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Pro Lys Thr Leu Met Ile Ser Arg Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

- 27 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ser Ala Val Gln Gly
1 5

5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

20

Val His Asn Ala Lys Thr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:15:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

40

Gly Cys Ala Glu Pro Trp Asn
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

45

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50

(v) FRAGMENT TYPE: internal

- 28 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Pro Leu Ala Phe

1

5

CLAIMS

1. A physiologically active molecule having an extended half-life in the circulatory system of a subject, the physiologically active molecule having a structure which is modified to include a specific amino acid sequence which binds to the IgG protection receptor FcRp but which does not bind to an Fc receptor which mediates immune effects.
2. The molecule of claim 1, wherein the specific amino acid sequence is substantially homologous to an amino acid sequence of at least a portion of the IgG domain which binds to the IgG protection receptor FcRp.
3. The molecule of claim 2, wherein the specific amino acid sequence is the same as the amino acid sequence of at least a portion of an IgG domain which binds to the IgG protection receptor FcRp.
4. The molecule of claim 1, which is a protein or peptide.
5. The molecule of claim 4, which is an immunoglobulin or portion thereof.
6. The molecule of claim 5, wherein the immunoglobulin or portion thereof is selected from the group consisting of all or a portion of IgG3, IgA, IgD, IgE, and IgM.
7. A physiologically active protein or peptide having an extended half-life in the circulatory system of a subject, the protein or peptide having an amino acid sequence which has been altered by amino acid substitution such that the protein or peptide specifically binds to the IgG protection receptor FcRp.
8. The molecule of claim 7, which is an immunoglobulin or portion thereof.
9. The molecule of claim 8, wherein the immunoglobulin or portion thereof is selected from the group consisting of all or a portion of IgG3, IgA, IgD, IgE, and IgM.
10. A physiologically active molecule having an extended half-life in the circulatory system of a subject, the physiologically active molecule having a structure which is modified to include at least a portion of an antibody which is raised against the IgG protection receptor FcRp and which specifically binds to the IgG protection receptor FcRp.

- 30 -

11. The molecule of claim 10, wherein the portion of the antibody is selected from group consisting of a Fab fragment, Fv fragment, Fab' fragment, and F(ab')₂ fragment.

5 12. The molecule of claim 10, wherein the portion of the antibody binds to the IgG protection receptor FcRp at acidic pH.

13. The molecule of claim 12, wherein the pH is about 6.5 or lower.

10 14. The molecule of claim 10, wherein the portion of the antibody does not bind to the IgG protection receptor at neutral pH.

15. The molecule of claim 14, wherein the pH is about 7.0 to about 7.5.

15 16. A composition comprising the molecule of claim 1 and a pharmaceutically acceptable carrier.

17. A composition comprising the molecule of claim 7 and a pharmaceutically acceptable carrier.

20 18. A composition comprising the molecule of claim 10 and a pharmaceutically acceptable carrier.

25 19. A method for producing a physiologically active molecule which has an extended half-life in the circulatory system of a subject, comprising modifying the structure of the molecule such that it includes a peptide which specifically binds to the IgG protection receptor FcRp.

30 20. The method of claim 19, wherein the peptide has an amino acid sequence which is substantially homologous to an amino acid sequence of at least a portion of the IgG domain which binds to the IgG protection receptor FcRp.

35 21. The method of claim 20, wherein the peptide has an amino acid sequence which is the same as the amino acid sequence of at least a portion of an IgG domain which binds to the IgG protection receptor FcRp.

- 31 -

22. The method of claim 19, wherein the physiologically active molecule is a protein or peptide.

23. The method of claim 22, wherein the protein or peptide is an immunoglobulin or portion thereof.

24. The method of claim 23, wherein the immunoglobulin or portion thereof is selected from the group consisting of all or a portion of IgG3, IgA, IgD, IgE, and IgM.

25. A method for producing a physiologically active protein or peptide having an extended half-life in the circulatory system of a subject, comprising altering the amino acid sequence of the protein or peptide by amino acid substitution such that the protein or peptide specifically binds to the IgG protection receptor FcRp.

26. The method of claim 25, wherein the protein or peptide is an immunoglobulin or portion thereof.

27. The method of claim 26, wherein the immunoglobulin or portion thereof is selected from the group consisting of all or a portion of IgG3, IgA, IgD, IgE, and IgM.

28. A method for producing a physiologically active molecule having an extended half-life in the circulatory system of a subject, comprising modifying the structure of the molecule such that it includes at least a portion of an antibody which is raised against the IgG protection receptor FcRp and which binds to the IgG protection receptor FcRp.

29. The method of claim 28, wherein the portion of the antibody is selected from group consisting of a Fab fragment, Fv fragment, Fab' fragment, and F(ab')₂ fragment.

30. The method of claim 28, wherein the portion of the antibody binds to the IgG protection receptor FcRp at acidic pH.

31. The method of claim 30, wherein the pH is 6.5 or lower.

32. The method of claim 28, wherein the portion of the antibody does not bind to the IgG protection receptor at neutral pH.

- 32 -

33. The method of claim 32, wherein the pH is about 7.0 to about 7.5.

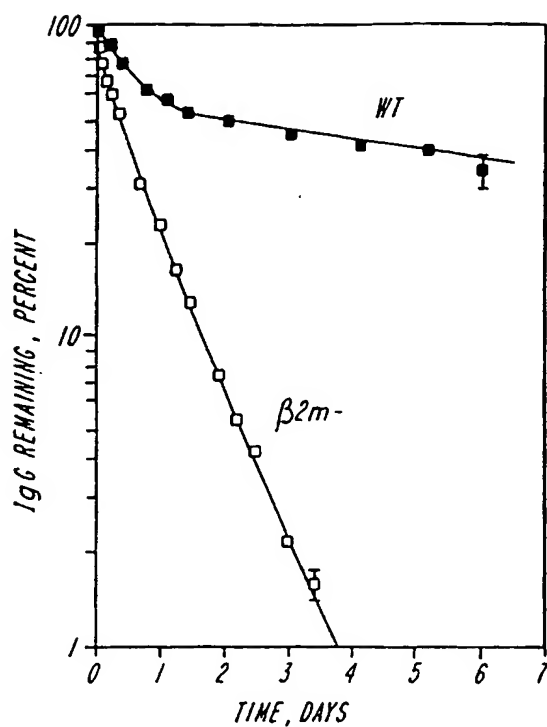
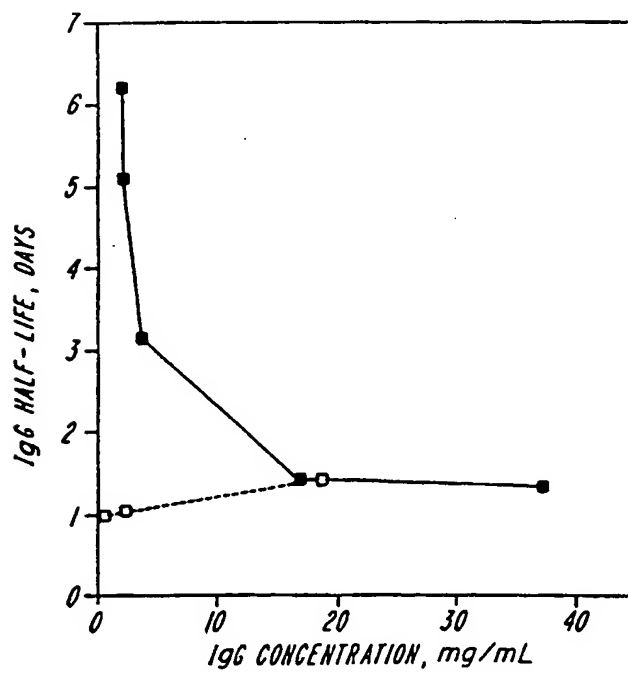
34. A method of treating a subject having a disorder which can be treated with a physiologically active molecule, comprising administering the composition of claim 16 to the
5 subject.

35. A method of treating a subject having a disorder which can be treated with a physiologically active molecule, comprising administering the composition of claim 17 to the
10 subject.

36. A method of treating a subject having a disorder which can be treated with a physiologically active molecule, comprising administering the composition of claim 18 to the
subject.

15 37. A physiologically active molecule having an extended half-life in the circulatory system of a subject, the physiologically active molecule having a structure which is modified to include a specific amino acid sequence which binds to the IgG protection receptor FcRp but which does not bind to complement.

1/2

**FIG. 1****FIG. 2**

2/2

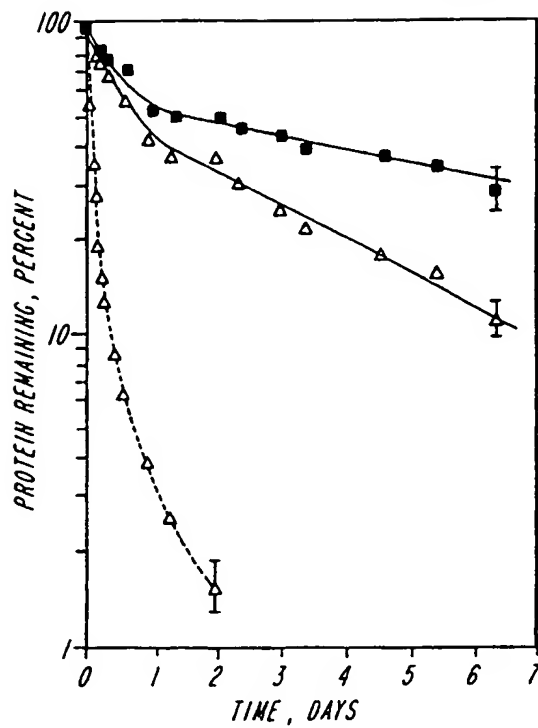


FIG. 3A

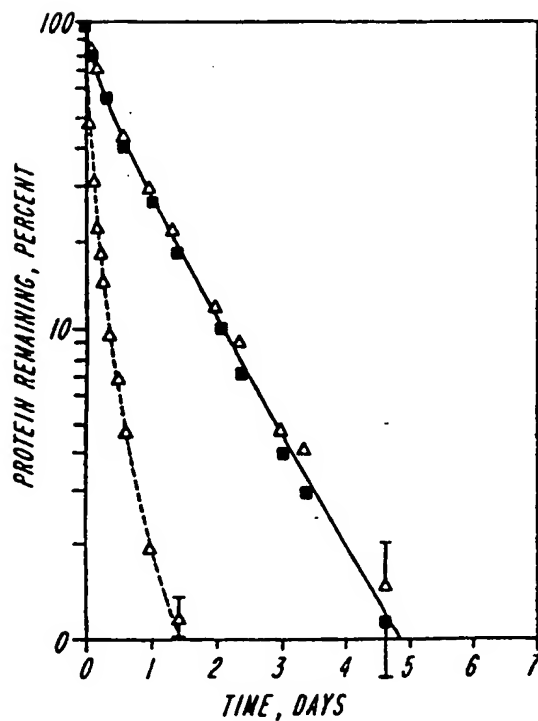


FIG. 3B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/07707

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 16/46; A61K 39/00, 39/395

US CL : 530/387.3, 387.9; 424/134.1, 139.1, 143.1, 192.1, 185.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.3, 387.9; 424/134.1, 139.1, 143.1, 192.1, 185.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JUNGHANS et al. The protection receptor for IgG catabolism is the B2-microglobulin-containing neonatal intestinal transport receptor. Proc. Natl. Acad. Sci. USA. May 1996, Vol. 93, pages 5512-5516, see the Abstract and page 5516.	1-37
A	JUNGHANS et al. The Brambell Protection Receptor (FcRp) for IgG catabolism is the neo-natal intestinal transport receptor (FcRn). FASEB Journal. 30 April 1996, Vol. 10, No. 6, Abstract No. 1737, see the entire abstract.	1-37
A	ISRAEL et al. Increased clearance of IgG in mice that lack B2-microglobulin: possible protective role of FcRn. Immunology. 1996, Vol. 89, pages 573-578. See the Abstract.	1-37

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* documents which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* A*	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

21 JUNE 1997

Date of mailing of the international search report

05 AUG 1997

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

TONI R. SCHEINER

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07707

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	Database Medline on Dialog, US National Library of Medicine, (Bethesda, MD, USA), No. 97200272, JUNGHANS, R.P. 'Finally! The Brambell receptor (FcRB). Mediator of transmission of immunity and protection from catabolism for IgG,' abstract, Immunologic Research, February 1997, 16(1), pages 29-57, see entire abstract.	1-37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07707

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS; DIALOG: file biochem

search terms: FeRp; FeRn; protection receptor; Brambell receptor; Brambell protection receptor; IgG; immunoglobulin; half-life; circulation; circulatory; clearance; catabolism

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.